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Please find below and/or attached an Office communication concerning this application or proceeding.

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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/720,940
Filing Date: January 02, 2001
Appellant(s): OSCHMANN ET AL.

William C. Gehris

For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed on 2/23/06 appealing from the Office action mailed on 6/21/05.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

No amendment after final has been filed.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is substantially correct. The changes are as follows: The 102 rejection is withdrawn.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Evidence relied upon by the examiner in the rejection of the claims under appeal as follows:

NAME	DATE	PATENT NUMBER
SCHWABE	12-2001	6,328,999
SCHWABE	04-1996	5,512,286
OSCHMANN	06-2002	6,399,099
SCHWABE	03-1995	5,399,348
SCHWABE	06-1994	5,322,688
YOSHIHARU	04-1994	JP 06279300: Translation provided

OTHER REFERENCES—NON-PATENT LITERATURE

LIU ZHENG et al, AN 1997:82596, HCAPLUS, abstract of Huaxue Shijie (1996), 37(7), 355-358. English translation provided.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Double Patenting – First Rejection

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 11-18 rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent No. 6,328,999. See also the entire document especially lines 7-45 in col. 1, lines 15-38 in col. 2, examples, and claims.. Although the conflicting claims are not identical, they are not patentably distinct from each other because the reference generically teaches the instant invention.

Instant claims differ from the reference in claiming a product-by-process by ultrafiltration[1] cut off range from 2,000 Daltons to 10,000 Daltons.

The reference teaches an extract from ginkgo biloba leaves comprising: 20 to 30% by weight flavonol glycosides a total of 2.5 to 4.5% by weight of ginkgolides A, B, C and J 2.0 to 4.0% by weight bilobalide below 10 ppm alkyl phenol compounds below 10% by weight proanthocyanidins below 50 ppm 4'-O-methylpyridoxine below 100 ppm biflavones, which generically teaches the instant invention.

The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process.*"

The process of ultrafiltration and cutoff range of 2,000 to 10,000 Daltons will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the same prior product. The compounds having cut off range less than 2,000 Daltons are taught by the prior art therefore, Applicant's claim of the extract containing cut off range 2000-10,000 would contain the compounds having molecular weight less than 2,000 and above 10,000. Most active compounds known from *Ginkgo biloba* have molecular weight less than 2,000. The Examiner for convenience has provided a list of compounds in Ginko biloba extract on Pages 12-14.

Double Patenting – Second Rejection

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

[1] Ultrafiltration membranes have molecular weight cut offs (MWCO's) ranging from 100 to 500,000 Daltons. The smaller than the rated MWCO of the membranes are capable of passing through the membrane.

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 11-18 rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-14 of U.S. Patent No. 5,512,286. See also the entire document especially lines 5-57 in col. 1, lines 13-39 in col. 2, lines 1-20 in col. 6, tables, examples, and claims. Although the conflicting claims are not identical, they are not patentably distinct from each other because the reference generically teaches the instant invention.

The reference teaches an extract from the leaves of Ginkgo biloba containing most of the flavone glycosides, ginkgolides and bilobalide originally present in the leaves, comprising 20 to 30 weight percent flavone glycosides, 2.5 to 4.5 weight percent ginkgolides selected from the group consisting of ginkgolide A, B, C and J and mixtures thereof, 2.0 to 4.0 weight percent bilobalide and less than 10 ppm alkylphenol compounds, said extract being essentially free of components of the leaves with serum-precipitating or hemagglutinating properties, which generically teaches in the instant invention.

The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process.*"

The process of ultrafiltration and cutoff range of 2,000 to 10,000 Daltons will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the prior product. See details in first rejection.

Double Patenting – Third Rejection

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right

to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 11-18 rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-25 of U.S. Patent No. 6,399,099. See also the entire document especially lines 28-67 of col. 1, lines 1-16 of col. 2, examples and claims. Although the conflicting claims are not identical, they are not patentably distinct from each other because the reference generically teaches the instant invention.

An effervescent composition for oral administration comprising a dry extract of ginkgo biloba comprising from 20-30% by weight of flavone glycosides and from about 4.5-8.5% by weight of terpenoids, which generically teaches the instant invention.

The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process.*"

The process of ultrafiltration and cutoff range of 2,000 to 10,000 Daltons will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the prior product. See details in first rejection.

Double Patenting – Fourth Rejection

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right

to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 11-18 rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-4 of U.S. Patent No. 5,399,348. See also the entire document especially lines 6-68 in col. 1,lines 1-10 in col. 2, lines 21-47 in col. 3, examples, and claims. Although the conflicting claims are not identical, they are not patentably distinct from each other because the reference generically teaches the instant invention.

The reference teaches an extract comprising 20 to 30 weight percent flavone glycosides, 2.5 to 4.5 weight percent of ginkogolides A, B, C and J, 2.0 to 4.0 weight percent bilobalide, less than 10 ppm alkylphenol compounds and less than 10 weight percent proanthocyanidins, which generically teaches the instant invention.

The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process*."

The process of ultrafiltration and cutoff range of 2000 to 10000 Daltons will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the prior product. See details in the first rejection.

Double Patenting – Fifth Rejection

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 11-18 rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 12 of U.S. Patent No. 5,322,688. See also the entire document especially lines 28-67 of col. 1, lines 1-16 of col. 2, examples and claims. Although the conflicting claims are not identical, they are not patentably distinct from each other because the reference generically teaches the instant invention.

The reference teaches an extract from the leaves of *Ginkgo biloba*, which is substantially free of alkylphenol compounds, and having a high content of flavone glycosides and comprising substantially all of the ginkgolides and bilobalide originally present in the leaves, which generically teaches the instant invention.

The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process.*"

The process of ultrafiltration and cutoff range of 2000 to 10000 Daltons will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the prior product. See details in the first rejection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> *(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.*

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 11-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over the following references:

1. Japanese Unexamined Patent Publication (A) No. 279300/1994 (Translation). Publication Date: 10/4/1994, The reference teaches a gingko leaf extract containing sufficient amounts of active ingredients and easily soluble in

water. The present invention also relates to a process for producing said extract. The reference teaches a water-soluble Ginkgo leaf extract containing at least 20% flavone glycoside and at least 5.6% terpene lactones. See the entire document, especially lines 1-11 on page 6, lines 3-19 on page 8, lines 1-13 on page 10, lines 13-25 on page 12, paragraph [0017] (Example 3) on page 18, paragraph [0018] on page 19, claims, and examples.

2. Liu, Zheng et al. (AN 1997:82596, HCAPLUS, abstract of Huaxue Shijie (1996), 37(7), 355-358). The references disclose a water-soluble extract containing total flavones from *Ginkgo biloba* leaves. See the entire English translation especially pages 4, Table 2 on page 5, the scheme and ***especially*** the water-soluble extract on page 6.

Both the references teach water-soluble extracts which embraces Applicant's claimed invention.

Instant claims differ from the reference in claiming an extract obtained by "ultrafiltration cut off range 2,000 to 10,000 Daltons".

It would have been obvious to one skilled in the art at the time of invention to prepare an extract as presently claimed, even if the prior art does not use the word "ultrafiltration" and the cutoff range of 2,000 to 10,000 Daltons, the claims have been subjected to other separation techniques, bringing the extract to similar ingredients/constituents as has been taught by the prior art. The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process.*" The process of ultrafiltration will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the prior product. It should be noted that the prior art does not teach the cutoffs of ultrafiltration of 2,000 to 10,000 Daltons. However, it teaches the water-soluble extract, which contains compounds of less than 2,000 Daltons, which is the same as the instant invention. Some structures of the compounds in *Gingko biloba* extract (GBE) having molecular weight less than 2,000 are listed on pages 12-14 of this action.

In the light of the forgoing discussion, the Examiner's ultimate legal conclusion is that the subject matter defined by the instant claims would have been obvious within the meaning of 35 U.S.C. 103(a).

(10) Response to Argument

The basis for the Applicants' arguments against all of the double patenting, 103, and 102 rejections is the same: that "the resultant product created by the ultrafiltration is different and novel over non-ultrafiltered Gingko leaf extracts, which contain particles much larger than 10,000 Daltons and do not consist essentially of the ultrafiltered particles as recited in claim 1."

The Examiner respectfully disagrees. The resultant product is *not* different because after the ultrafiltration and the cutoff range of 2,000 to 10,000 Daltons, the filtrate contains the compounds having molecular weight of less than 2,000 Daltons. The compounds having molecular weight of less than 2,000 Daltons include many active compounds present in *Gingko biloba* extract (already on the market), such as flavones, flavonglycosides, terpenlactones, and bilobalide.

The Applicants have claimed a product-by-process. The MPEP, in section 2113, states: "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is ***unpatentable*** *even though the prior product was made by a different process.*"

The process of ultrafiltration will produce a product that is obvious over the prior art. The claim is unpatentable, even though a different process made the prior product.

The Applicants also argue for nearly every rejection: " prior art does not teach or show 'wherein the extract is produced by ultrafiltration using a filter having an average molecular weight cut off ranging from 2,000 to 10,000 Daltons' as recited in claim 11 and thus the rejection."

The Examiner's position is that **THE 'AVERAGE MOLECULAR WEIGHT CUT OFF RANGE' IS *NOT* a DISTINGUISHABE CRITERIA OR PROPERTY IN THIS CASE. THE APPLICANTS ARE CLAIMING THE PRODUCT, NOT THE *PROCESS* TO GET THE PRODUCT. IF THE APPLICANTS CLAIMED ANY DALTONS CUT OFF RANGE, IT STILL WOULD BE OBVIOUS BECAUSE THE APPLICANTS ARE CLAIMING THE PRODUCT, NOT THE PROCESS.**

It does *not* matter what process the Applicants went through to get the product because the products containing compounds of less than 2,000 are the same. The Applicants are claiming the product, **NOT** the process.

The extract of the present invention (Claim 11) contains the compounds of less than 2,000 molecular weight because the cutoff is 2,000 to 10,000 Daltons. The compounds, which are present in the prior art extract, have molecular weight less than 2,000 or more than 10,000 will ***be the same***.

The following are compounds present in *Gingko biloba* water-soluble extracts (GBE). Please note that the molecular weight of these compounds is less than the cut off range of 2,000. These compounds will be present in the product as presently claimed.

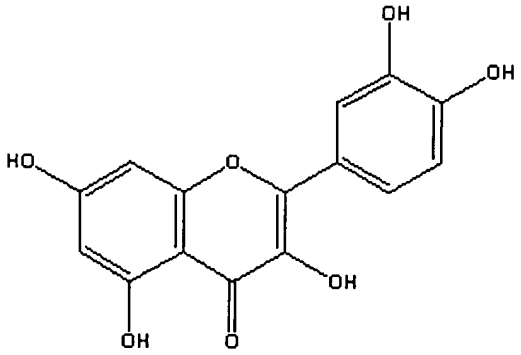
Standardized ingredients of GBE

Common Name:Quercetin

CAS Registry Number:117-39-5

Chemical Abstracts Service Name:4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)- 3,5,7-trihydroxy-(9CI)

Structure, Molecular Formula and Molecular Weight:



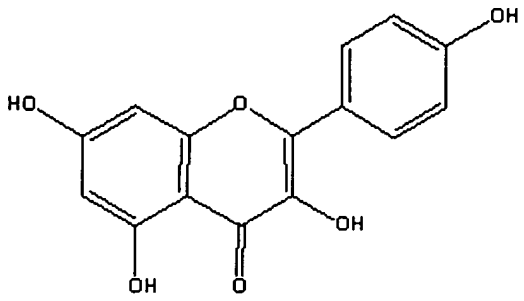
$C_{15}H_{10}O_7$ Mol.wt.: 338.3

Common Name:kaempferol

CAS Registry Number:520-18-3

Chemical Abstracts Service Name: 4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-(4- hydroxyphenyl)-(9CI)

Structure, Molecular Formula and Molecular Weight:



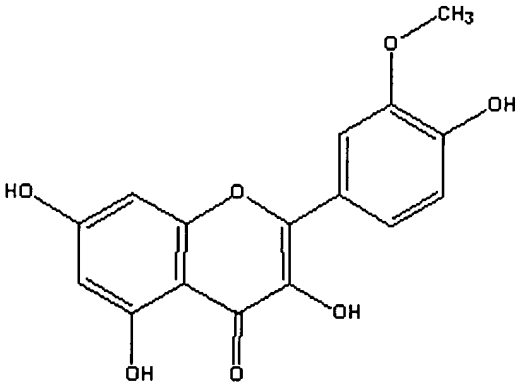
C_{15} H_{10} O_6 Mol.wt.: 286.2

Common Name:Isorhamnetin

CAS Registry Number:480-19-3

Chemical Abstracts Service Name: 4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-(4- hydroxy-3-methoxyphenyl)-(9CI)

Structure, Molecular Formula and Molecular Weight:

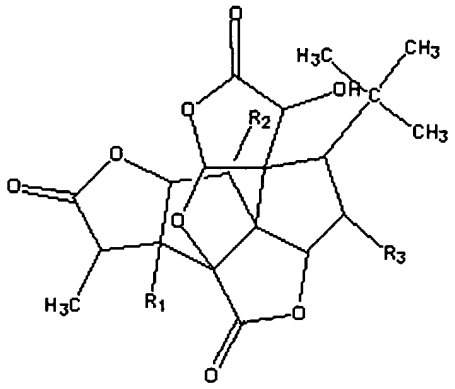


C_{15} H_{12} O_6Mol. wt.: ~314

Common Name:Ginkgolides (mixed); ginkgolide A; ginkgolide B

CAS Registry Number: 15291-77-7; 15291-75-5; 15291-75-5

Structure, Molecular Formula and Molecular Weight:



C_{20} H_{24} O_{10} (Ginkgolide B) Mol. wt.: 424.4 (Ginkgolide B)

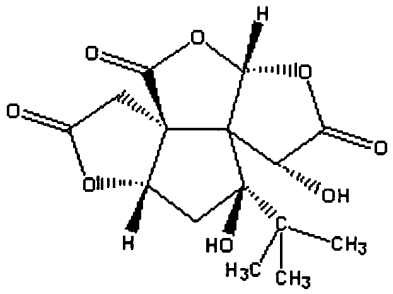
	R1	R2	R3
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH
Ginkgolide M	H	OH	OH

Common Name: Bilobalide

CAS Registry Number:33570-04-6

Chemical Abstracts Service Name:4H,5aH,9H- Furo(2,3-b)furo(3',2':2,3)cyclopenta (1,2-c)furan-2,4,7 (3H,8H) - trione 9-(1,1-dimethylethyl)-10,10a-dihydro-8,9-dihydroxy-, (5aR-(3aS*,5aα,8β,8aS*,9α,10aα))- (9Cl)

Structure, Molecular Formula and Molecular Weight:



$C_{15}H_{18}O_8$Mol. wt.: 326.3

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

SABIHA QAZI, PH.D
PRIMARY EXAMINER

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PTO 2006-4609

Chinese Article

Study on the water extract method of total flavonoids of Ginkgo biloba leaves

[银杏叶总黄酮水浸提法研究]

Liu Zheng, Chen Yong—le

UNITED STATES PATENT AND TRADEMARK OFFICE

Washington, D.C. [Month year]

Translated by: Schreiber Translations, Inc.

Translated Title	:	**Study on the water extract method of total flavonoids of Ginkgo biloba leaves**
Foreign Language Title	:	银杏叶总黄酮水浸提法研究
Authors	:	Liu Zheng, Chen Yong—le
Author Affiliation	:	Department of Applied Chemistry, Guilin Institute of Technology
Source	:	Huaxue Shijie

Study on the water extract method of total flavonoids of Ginkgo biloba leaves

Liu Zheng Chen Yong-le
(Department of Applied Chemistry, Guilin Institute of Technology)

Abstract: The water extract method of total flavonoids of Ginkgo biloba leaves is not so popular due to the low content of flavonoids, though the method really has its own well-known merits. This paper endeavors to find the best operation conditions to extract flavonoids from Ginkgo biloba leaves using a method known as orthogonal design. We then performed research on different methods that would yield a high probability of raising the content of flavonoids. We finally found out that the most effective method to increase the flavonoid content in the water extract was to take turns to perform several extraction and then chromatography refinement.

Key words: flavonoids; orthogonal design; water

1. Introduction

In today's world, health food, including the oral solution of Ginkgo biloba leaves and granules made from Ginkgo biloba leaves, has become very fashionable all over the world, including countries such as South Korea, Germany, France, Sweden, etc. In recent years, we have seen the rapid expansion of the Ginkgo biloba food market on the domestic front. Yet, in terms of Ginkgo biloba leaves R&D and utilization efforts, we are still far behind some of the other developed countries. For too long we have been stuck with only concerning ourselves about the exportation of Ginkgo biloba leaves. This has led to a real waste of national resources. Therefore, at this stage, it is very important to pay much more attention to the areas of Gingko biloba leaves R&D and utilization now.

At present, the most commonly-used solvents to extract water from the total flavonoids of the Ginkgo biloba leaves include water, ethanol and methanol. It costs the least to use water extract. According to our investigation of local enterprises in Guilin, the total flavonoids content level remains around 10% when using water as solvent. Some pharmacy plants and study institutions purchase a large amount of flavonoids ointment from the areas that produce the Ginkgo biloba (e.g. Xing'an county), and demand a content level around16%. But due to technology problems, most local enterprises are unable to meet this demand. This results in lower prices and makes it difficult to even close a deal. If one wants to replace the water with ethanol or other organic solvents, this will require a one-time investment that is too cost-prohibitive for most local enterprises. One of our main purposes is therefore to help local enterprises resolve the technology problems associated with the water extract method.

2. Experiment

2.1 Reagent and equipment

IB801 super Constant Temperature Ovens: Constant Temperature Ovens instrument factory in Liaoyang city;

SHZ-B versatile water-cycle vacuum pump: YingYu instrument factory district Gong Henan province;

72 type spectrophotometer: analytical instrument factory in Shanghai;

30% ethanol; 1mol/L NaOH

Solution of $NaNO_2$ (1:20): weight $NaNO_2$ 5.000g (analytical purity) precisely and dissolved in a 100mL beaker. Feed the solution into a 100ml volumetric flask and add water to the standard ring.

2.2 Analysis of flavone

Rutin is used as a standard solution to measure total flavone (the maximum wavelength in this experiment is 500nm. The working curve is $Y(g/L) = 0.07808A + 0.0001943$, in which A is absorbance). This method follows: weight certain amount of flavone and put it into a 25 ml colorimeter tube, add ethanol to 12.5 ml. Add 0.7ml $NaNo_2$ (1:20), shake the solution evenly and add 0.7 ml Al $(OH)_3$ (1:10) into it. Add 5ml 1mol/L NaOH into it again after 6min and mix it evenly. Dilute the solution and make the fluid level to the graduation mark by using 30% of ethanol. Take the reagent as blank 10 min later and use 1cm colorimetric cell to determine the absorbance value at 500 nm. Check the concentration of the flavones solution from its working curve. Then figure out the percentage yield and the total contents of flavones according to the weight of the end product after baking the leaching liquor.

3 Results and discussion

3.1 Determination of the optimal condition of the extraction process

We carried out orthogonal design experiment to ensure the best extraction conditions. The factors and levels are given in table 1.The results of the experiment are shown in table 2. The method of the experiment is shown below: Weigh 10.0g shredded leaves of maidenhair tree and bake them for 1.5 h under the temperature of 110°C. Fix the volume of the solution at 250ml after extracting experiment-using water as solvent and concentrate the liquid under reduced pressure. Take out 1 ml liquid, determine its absorbance and figure out the percentage yield and content using method 2.2 at 500nm.

Table 1 Orthogonal test table with factors and levels

Factors / els	Water to material ratio	Degree of temperature (°C)	Time (h)
1	20:1	80	8
2	30:1	85	11
3	40:1	90	14

The orthogonal design experiments take the contents of the flavones as the main inspection standard. It can be seen from table 2 that the optimal condition $A_3B_2C_2$ obtained by calculation and analysis contradicts with the optimal condition resulting from direct analysis. Doing the experiment one more time, the absorbance value we obtained on $A_3B_3C_2$ (0.325) is greater than that on $A_3B_3C_3$ (0.310) which indicates that the condition based on calculation and analysis has an advantage of that obtained by direct analysis. Therefore, we conclude the optimal condition for using water as a solvent to extract is as follows: water to material ratio is 40:1, extracting temperature is 90°C and the extracting time is 11h. According to the grade difference, the orders of these effects are: B>A>C---that is to say the temperature has the most powerful influence on the contents of the extraction.

Table 2: Results analysis table of orthogonal test

Factors / Row / Num	Ratio between water and material A	Temperature B (°C)	Time C (h)	Absorbance	Proportion %	Content %
	1	2	3			
1	1(20:1)	1(80)	3(14)	0.180	0.89	5.24
2	2(30:1)	1	1(8)	0.229	1.13	4.52
3	3(40:1)	1	2(11)	0.267	1.32	4.40
4	1	2(85)	2	0.257	1.27	4.70
5	2	2	3	0.257	1.27	4.70
6	3	2	1	0.252	1.24	4.20
7	1	3(90)	1	0.285	1.40	5.19
8	2	3	2	0.299	1.47	5.26
9	3	3	3	0.310	1.53	5.46
I	0.722	0.676	0.786			I=2.336
II	0.785	0.766	0.823			
III	0.829	0.894	0.747			
R	0.107	0.218	0.076			

3.2 The determination of the number of extraction experiment

The purpose of this test is to determine the number of extractions, in which water is used as a solvent, with optimal extraction time of 11 hours, temperature of 90°C and 10g leaves of maidenhair tree. The data analysis is given in table 3.

From table 3, we can see that the fourth extraction has a lower yield compared with the first time. We choose 3 times as the optimal extraction condition----take the production cost and time into consideration. In addition, the flavones obtained from the test in multi-times extraction experiment are 7.07%, which are higher than that in one-time extraction.

3.3 Alternate tests

For further increasing the contents of flavones, we make reference to reference [1].

entry	water (ml)	time (h)	Analysis condition(ml)		absorbency	yield%	total yield%	total content %
			Constant volume	Sample amount				
1	400	5.5	250	1	0.325	1.60	2.05	7.07
2	250	2.5	150	3	0.288	0.28		
3	200	2	100	4	0.216	0.11		
4	150	1	50	6	0.355	0.06		

This paper designed three batches of alternative twice extraction experiments with water as the solvent. These steps are as follows:

4g Ginkgo Biloba dry → dry leaf → 100ml water → extract liquid filter → extraction for 3h →

filtrate1a (reserve)

residue → 100ml water → extract liquid filter → Filtrate 1b → Extraction for 2h →

residue

(discard)

5g Ginkgo Biloba dry → dry leaf → 1b → extract liquid filter → extraction for 2h →

filtrate3a(reserve)

residue → 100ml water → extract liquid filter → filtrate 1b → extraction for 1h →

residue

(Discard)

5g Ginkgo Biloba dry → dry leaf → 2b → extract liquid filter → extraction for 2h →

filtrate3a (reserve)

residue → 100ml water → extract liquid filter → filtrate 3b → extraction for 1h → (reserve)

residue

(Discard)

Finally, intermix filtrates 1a, 2a, 3a, 3b and decompress, concentrate to less than 250ml; pour into 250ml flask for constant volume; test the absorbency; decompress and concentrate again; dry and then obtain product, weight. The results of the experiments are shown in table 4

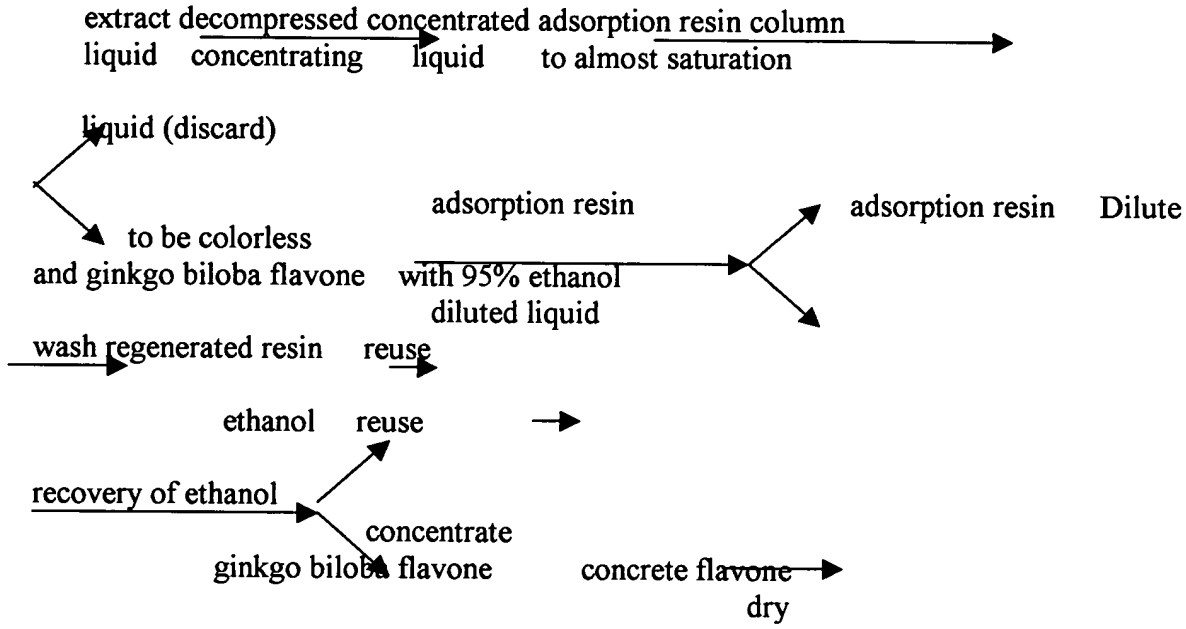
Table 4 Results of the alternative experiments

solvent	absorbency A	weight Gg	yield%	content%
water	1.00	2.7	4.89	18.1

As shown in table 4, these alternative experiments are highly effective methods for improving and raising Flavone content.

3.4 Chromatography Purification

In order to improve flavone content, column chromatography is used to purify flavone. Polyamide resin is used as an adsorption resin while ethanol is used as a diluting solvent. The extract liquid is obtained by 3 batches of alternative twice extraction with 60% ethanol-water solvent; the steps are similar to 3.3. It is described as follows:



The experiment results shown in table 5 indicate that refining will cause some loss of flavone and lead to low yield but high purity.

Table 5 Data of alternative extract liquid chromatography purification

Solvent	Absorbency A	Weight Gg	Yield%	Content%
60% ethanol-water (three batch of alternative twice extraction)	0.315	0.7	1.55	22.1

4 Conclusions

This paper presents a detailed study about a water extraction method that is most commonly used and costs the least. It discusses the most ideal condition of water extraction for flavone and concluded that chromatography purification after several times alternative extraction is the most effective way to improve flavone content.

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Date: 1995.3.29

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Article

Research and application of producing pulp-mold double membranes transparent glue with SBS

[SBS 制取纸塑复膜透明胶粘剂的研制和应用]

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[Title of the Invention] Water-soluble ginkgo leaf extract and process for producing the same

[Abstract]

[Object] Providing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones at a remarkably reduced cost.

[Constitution] A water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, the extract being obtained by concentrating and drying a ginkgo leaf extract solution adjusted to pH 5.0 to 7.0. A process for producing the same comprises adding a water-insoluble ginkgo leaf extract extracted with a water-containing organic solvent to water or a water-containing organic solvent, adding a basic compound to the solution [sic] at 30°C or less to adjust the pH to 6.0 to 7.0, filtering the mixture, and concentrating and drying the filtrate. Another process for producing the same comprises adding a water-insoluble ginkgo leaf extract to water or a water-containing organic solvent, adding a basic compound to the solution [sic] at 80°C or less to adjust the pH to 5.0 to 6.0, filtering the mixture, and concentrating and drying the filtrate.

[Claims]

[Claim 1] A water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, the extract being obtained by concentrating and drying a ginkgo leaf extract solution adjusted to pH 5.0 to 7.0.

[Claim 2] A process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, the process comprising adding a water-insoluble ginkgo leaf extract extracted with a water-containing organic solvent to water or a water-containing organic solvent, adding a basic compound to the solution [sic] at 30°C or less to adjust the pH to 6.0 to 7.0, filtering the mixture, and concentrating and drying the filtrate.

[Claim 3] A process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, the process comprising adding a water-insoluble ginkgo extract extracted with a water-containing organic solvent to water or a water-containing organic solvent, adding a basic compound to the solution [sic] at 80°C or less to adjust the pH to 5.0 to 6.0, filtering the mixture, and concentrating and drying the filtrate.

[Claim 4] A process for producing a water-soluble ginkgo

leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that in the step of obtaining a water-insoluble ginkgo leaf extract, a basic compound is added at 30°C or less to prepare a solution having a pH of 6.0 to 7.0, which is then filtered, concentrated and dried.

[Claim 5] A process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that in the step of obtaining a water-insoluble ginkgo leaf extract, a basic compound is added at 80°C or less to prepare a solution having a pH of 5.0 to 6.0, which is then filtered, concentrated and dried.

[Claim 6] A process for producing a water-insoluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that in the process of preparing a water-insoluble ginkgo leaf extract as defined in claim 4 or 5, the following procedures (i) to (iv) are sequentially carried out:

(i) extracting dry ginkgo leaves with an aqueous solution containing 40 to 80% of ethanol with heating,

(ii) concentrating the extract obtained in (i) to half or less of its original volume and cooling and filtering the concentrate,

(iii) bringing the filtrate obtained in (ii) into contact

with a substituent-free porous resin to adsorb the ginkgo leaf extract to the resin, washing the porous resin with water, and bringing an aqueous solution containing at least 60% of ethanol into contact with the washed porous resin to desorb the extract from the resin, or successively bringing an aqueous solution containing 10 to 40% of ethanol and an aqueous solution containing at least 60% of ethanol into contact with the washed porous resin to gradually desorb the ginkgo leaf extract from the resin, and

(iv) concentrating and drying the eluate obtained by the desorption in (iii) to obtain an extract.

[Detailed Description of the Invention]

[0001]

[Field of the Invention] The present invention relates to a ginkgo leaf extract containing sufficient amounts of active ingredients and easily soluble in water. The present invention also relates to a process for producing said extract.

[0002]

[Prior Art and Problems to be Solved by the Invention] In recent ten and several years, therapeutic compositions predominantly comprising a ginkgo leaf extract extracted with a water-containing organic solvent have been widely used in Germany, France and other countries as medicament

for the purpose of ameliorating the cerebral artery and peripheral vessel. It is known that a ginkgo leaf extract contains, as active ingredients, flavonoid glycoside as well as terpene lactones, which are components peculiar to Ginkgo, i.e., Ginkgolide A, B, C and J and Bilobalide. It is clinically recognized that a ginkgo leaf extract ameliorates blood circulation without rise in blood pressure and has therapeutic effects on senile dementia, Raynaud disease which is a peripheral vessel disorder, and various disorders caused by diabetes. Further, the extract has active enzyme removal effects, and antiallergic effects which were recognized recently. Moreover, the extract causes substantially no adverse effects. Therefore, a ginkgo leaf extract is on the market and coming into wide use, not only as a medicine but also as health food.

[0003] However, a ginkgo leaf extract extracted with a water-containing organic solvent has a serious drawback that the extract is insoluble in water. Thus, the extract has been generally used as a tincture prepared by dissolving the extract in a 50% aqueous solution of alcohol, solid preparations obtained by tabletting a powdery extract as such, and the like.

[0004] In recent years, however, processes have been proposed to make the ginkgo leaf extract soluble in

water. Such processes for producing a water-soluble ginkgo extract is, for example, (1) a process comprising extracting dry ginkgo leaves with a water-containing organic solvent or the like, adjusting the extract to pH 7 to 10, preferably pH 7.5 to 9, and passing the extract through a column filled with a nonpolar porous resin for purification. Also proposed are (2) processes for extracting and purifying an effective bioactive substance in ginkgo leaves. One of such processes comprises extracting ginkgo leaves with a strongly alkaline aqueous solution (a saturated calcium hydroxide solution), and another process comprises adjusting the extract to pH 6 to 8 during purification. Further proposed is (3) a process comprising adjusting a ginkgo leaf extract extracted with a water-containing organic solvent to strong alkalinity of pH 8.0 or more, preferably pH 14, readjusting the extract to acidity of pH 1 for removal of undesired substances (protoanthocyanidins), and then carrying out the next procedure.

[0005] However, all of the proposed techniques have serious problems that the obtained ginkgo leaf extract does not contain a sufficient amount of terpene lactones which are important active ingredients of a ginkgo leaf extract. Specifically stated, in the process (2), ginkgo leaves are subjected to extraction with a strongly

alkaline solution for a prolonged period of time, and the processes (1) and (3) comprises the step of adjusting the pH to a strong alkalinity from pH 7.5. In these processes, a large amount of terpene lactones in the extract is decomposed, since the extract is treated at a high pH value. In the process (2), although the amount of decomposed terpene lactones is smaller than in the other processes, the amount of terpene lactones in the resulting ginkgo leaf extract is as small as about one third of that in the extract prepared by the process of the present invention. Therefore, a technique for further purifying the ginkgo leaf extract obtained by the process (2) is also disclosed. However, the technique necessitates prolonged steps, involves complicated procedures and gives the purified product in a low yield. Further, the technique has a problem that it uses organic solvents which are not usable for food (e.g., butanol and ethyl acetate) and harmful matters such as bismuth compounds.

[0006] The present inventors conducted extensive research to solve the above problems and successfully obtained a water-soluble ginkgo leaf extract containing sufficient amounts of the both active ingredients, i.e., flavonoids and terpene lactones. Thus, an object of the present invention is to provide a method capable of easily

obtaining a water-soluble ginkgo leaf extract containing sufficient amounts of active ingredients. Another object of the present invention is to provide a ginkgo leaf extract containing sufficient amounts of active ingredients which is readily applicable to a wide variety of fields, for example, pharmaceutical compositions such as injection, materials for food and drink, materials for cosmetics, and the like.

[0007]

[Means for Solving the Problems] The present inventors conducted various experiments and found that, in the ginkgo leaf extract which is made soluble in water by pH adjustment, the amount of terpene lactones is closely related with the adjusted pH and the temperature of the solution during adjustment, and that terpene lactones are markedly decomposed if the solution is adjusted to pH 7 or more, and the temperature of the solution has an unexpectedly large influence. The inventors further found that the pH is adjusted to an unnecessarily large value in the prior art techniques, and that when the pH is adjusted to about 5, the extract is fully soluble in water and undesired substances, i.e., protoanthocyanidins can be removed therefrom to a good extent. The present invention has been accomplished based on these findings.

[0008] Thus, the present invention provides (1) a water-

soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, the extract being obtained by concentrating and drying a ginkgo leaf extract solution adjusted to pH 5.0 to 7.0, (2) a process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that the process comprises adding a water-insoluble ginkgo extract extracted with a water-containing organic solvent to water or a water-containing organic solvent, adding a basic compound to the solution [sic] at 30°C or less to adjust the pH to 6.0 to 7.0, filtering the mixture, and concentrating and drying the filtrate, (3) a process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that the process comprises adding a water-insoluble ginkgo extract extracted with a water-containing organic solvent to water or a water-containing organic solvent, adding a basic compound to the solution [sic] at 80°C or less to adjust the pH to 5.0 to 6.0, filtering the mixture, and concentrating and drying the filtrate, (4) a process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that in the step of

obtaining a water-insoluble ginkgo leaf extract, a basic compound is added at 30°C or less to prepare a solution having a pH of 6.0 to 7.0, which is then filtered, concentrated and dried, (5) a process for producing a water-soluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that in the step of obtaining a water-insoluble ginkgo leaf extract, a basic compound is added at 80°C or less to prepare a solution having a pH of 5.0 to 6.0, which is then filtered, concentrated and dried,

[0009] and (6) a process for producing a water-insoluble ginkgo leaf extract containing at least 20% of flavone glycoside and at least 5.6% of terpene lactones, characterized in that in the process for preparing the water-insoluble ginkgo leaf extract according to (4) or (5) above, the following procedures (i) to (iv) are sequentially carried out:

(i) extracting dry ginkgo leaves with an aqueous solution containing 40 to 80% of ethanol with heating,

(ii) concentrating the extract obtained in (i) to half or less of its original volume and cooling and filtering the concentrate,

(iii) bringing the filtrate obtained in (ii) into contact with a substituent-free porous resin to adsorb the ginkgo

leaf extract to the resin, washing the porous resin with water, and bringing an aqueous solution containing at least 60% of ethanol into contact with the porous resin to desorb the extract from the resin, or successively bringing an aqueous solution containing 10 to 40% of ethanol and an aqueous solution containing at least 60% of ethanol into contact with the washed porous resin to gradually desorb the ginkgo leaf extract from the resin, and

(iv) concentrating and drying the eluate obtained by the desorption in (iii) to obtain an extract.

[0010] The present invention is specifically described below. A ginkgo leaf extract extracted with a water-containing organic solvent is usually acidic and has a pH of about 3.0 to 4.5. Therefore, the extract is dispersed in water or dissolved in an aqueous solution of an organic solvent, and a basic compound such as an aqueous solution of sodium hydroxide is added to adjust the dispersion or solution to pH 5.0 to 7.0. It is preferred to adjust the pH at 30°C or less and especially preferred pH is 5.0 to 6.0. The water or the aqueous solution of an organic solvent containing the ginkgo leaf extract adjusted to pH 5.0 to 7.0 contains a small amount of undesired substances (protoanthocyanidins) precipitated and insoluble in water. Therefore, the solution is

filtered to remove the undesired substances and then concentrated and dried, giving the desired water-soluble ginkgo leaf extract. The process of the present invention gives a water-soluble ginkgo leaf extract in a yield of substantially 100% only by adjusting the pH, without necessitating any other complicated procedures.

[0011] In the process of the present invention, the pH can be adjusted during purification of a water-insoluble ginkgo leaf extract extracted with a water-containing organic solvent. In this case, it is preferred to adjust the pH before concentration, i.e., the last step of the purification. According to the present invention, the basic compound for adjusting the pH is a compound showing basicity, such as hydroxides, carbonates, bicarbonates, phosphates and methaphosphates of alkali metals and alkali earth metals. Also usable are, for example, ammonium salts such as ammonium hydrogencarbonate and ammonium citrate, alkylamines, polyalkylamines, aqueous ammonia and the like. In any event, the basic compound for use in the present invention is not limited to those exemplified above insofar as it shows basicity. Experiments revealed that weakly basic compounds are preferably used among these basic compounds.

[0012] In the above description, "a water-insoluble ginkgo leaf extract" means a solid product prepared by

extracting ginkgo leaves with a water-containing organic solvent such as water-containing methanol, water-containing ethanol, water-containing acetone, water-containing methyl ethyl ketone or the like, and subjecting the obtained extract to a combination of treatments such as concentration, filtration, treatment with a column, re-extraction with an organic solvent, and the like. The extract contains sufficient amounts of flavonoid glycoside and terpene lactones as active ingredients, and 1 g of the extract is not dissolved in 100 ml of water.

[0013]

[Examples]

The invention is further illustrated in detail by the following examples.

Example 1: A 10 g quantity of a commercially available water-insoluble ginkgo leaf extract containing 24.9% of flavone glycoside and 6.5% of total terpene lactones was dispersed, with stirring, in 50 ml of purified water at ambient temperature. To this dispersion was gradually added dropwise a 10% aqueous solution of potassium hydrogencarbonate to adjust the pH to 6.3. During this step, the water-insoluble ginkgo leaf extract dispersed was dissolved. When stirring was halted, a small amount of insoluble matter was observed. Then 5 g of a filter

aid (trade name: Highflosupercell(?)) was added and the solution was subjected to suction filtration. The resulting filtrate was concentrated under reduced pressure to give 9.8 g of a brown powder. Yield was approximately 100% upon moisture correction. 1 g of the brown powder thus obtained was dissolved in 100 ml of water, giving a transparent solution having a pH of 6.0. In the similar manner, additional powders having pH 7.3, pH 6.9, pH 6.6, pH 6.4, pH 5.6, pH 5.2 and pH 4.9 were prepared by controlling the amounts of a 10% aqueous solution of potassium hydrogencarbonate added.

[0014] Example 2: The procedure of Example 1 was repeated except that during the pH adjustment, the dispersion of ginkgo leaf extract was heated to 80°C for dissolution and then an aqueous potassium hydrogencarbonate was added, giving the ginkgo leaf extracts having pH 5.2, pH 6.0, pH 6.6, pH 6.9 and pH 7.3. Then, ginkgo leaf extracts having different values of pH prepared by the above steps in which reaction temperatures were different were checked for the contents of flavone glycoside and terpene lactones. To quantify flavone glycoside, extract samples were hydrolyzed by HCl in advance and applied to high performance liquid chromatography (HPLC) equipped with a C-18 reversed phase column containing silica gel as a carrier. A mixture of a 0.5% aqueous citric acid

solution/acetonitrile/isopropanol was used as eluent and then UV absorbance was measured. Commercially available quercetin and kaempferol were used as standards and calculation was performed by multiplying the measured values by a glycoside calculating coefficient which was determined based on molecular weights. As for terpene lactones, quantification was conducted by means of HPLC equipped with the same columns as used with flavone glycoside, using ether fractions obtained by thorough extraction with ether. More specifically, a differential refractometer was employed for the measurement using a mixture of water/methanol as eluent. The standards used were gingkolide A, B, C and bilobalide, all of which were isolated by HPLC under the same conditions, and the results were expressed in terms of the total values of the above four compounds. The results of measurements were shown in Table 1.

[0015]

[Table 1]

	pH adjustment at room temperature			pH adjustment at 80°C		
pH of extract	Content of total terpene lactones (%)	Decomposition ratio during process	Content of flavone glycoside (%)	Content of total terpene lactones (%)	Decomposition ratio during process	Content of flavone glycoside (%)
7.3	5.1	21.5	25.5	1.9	71	24.8
6.9	6.1	6.2	25.2	2.8	57	25.1
6.6	6.2	4.6	24.7	4.4	32.3	24.9
6.4	6.2	4.6	25.3			
6.0	6.5	0	25.0	5.9	9.2	25.1
5.6	6.4	0	24.4			
5.2	6.5	0	25.1	6.1	7.2	25.1
4.9	6.5	0	25.1			

[0016] As shown in Table 1, all extract samples obtained under different kinds of conditions contained sufficient amounts of flavone glycoside, whereas terpene lactones are significantly influenced by the pH values and reaction temperatures, resulting in the big difference in the amount of the remaining terpene lactones. That is to say, at a pH of 6 or less, the decomposition ratio is suppressed to the minimum (10% or lower) even if pH adjustment is conducted with heating. When the pH adjustment is conducted at low temperatures, the decomposition ratio is suppressed to the minimum up to a

pH of 7. Preferably pH was adjusted to 5 - 6 at low temperatures. 1 g of the sample having a pH of 4.9 was not soluble in 100 ml of water thoroughly and unable to make a transparent solution.

[0017] Example 3: To 500 g of coarsely pulverized dry ginkgo leaves was added 500 ml of a 75% aqueous solution of ethanol, and the mixture was heated at 55°C for 2 hours. This procedure was repeated three times. Then solid-liquid separation was performed by means of suction filtration. The liquid thus obtained was concentrated under reduced pressure until the volume was reduced to 500 ml. To the resulting liquid was added 500 ml of water, and the mixture was cooled to room temperature and then filtered, thereby removing hydrophobic substances precipitated. Then the obtained filtrate was passed through a glass column packed with 500 ml of "Diaion HP20" (trade name; a nonpolar porous resin produced by Mitsubishi Kasei Kogyo Kabushiki Kaisha) to adsorb the ginkgo leaf extract. The packing material, to which ginkgo leaf extract was adsorbed, was washed with 1000 ml of water, and then 1000 ml of a 70% aqueous solution of ethanol was supplied to desorb and elute the adsorbed extract. The eluate was adjusted to pH 6.0 by the addition of a 10% aqueous solution of ammonium hydrogencarbonate. Then the solution was filtered to

remove insoluble proanthocyanidin, and the filtrate was concentrated and dried under reduced pressure to give 21.0 g of a brown, water-soluble ginkgo leaf extract powder containing 25% flavone glycoside and 6.7% terpene lactones.

[0018] Comparative Example 1: A 500 g quantity of dry ginkgo leaves was immersed in 6000 ml of water and extraction was performed at 80 - 90°C for 2 hours. Ginkgo leaves were filtered off and to the resulting filtrate a 10% aqueous solution of NaOH was added to adjust the pH to 7.5. The filtrate was filtered again, and the filtrate was applied on the column packed with 500 ml of "Diaion HP20" for adsorption. After washing with 1500 ml of water, 1000 ml of a 60% aqueous solution of ethanol was supplied, to thereby desorb and elute the adsorbate. Evaporation of the resultant liquid to dryness under reduced pressure gave 15 g of a powder. 1 g of this ginkgo leaf extract was dissolved in 100 ml of water, giving a transparent solution. However, quantification revealed that this extract contained 21 % of flavone glycoside and only 1.3% of terpene lactones.

[0019] Comparative Example 2: A 100 g quantity of dry ginkgo leaves was extracted with 1000 ml of butyl alcohol/ethyl acetate mixture (3 : 1) at 80°C for 8 hours. This procedure was repeated three times. Then

the ginkgo leaves were filtered off and the resulting filtrates were combined and concentrated under reduced pressure, thereby giving a solid. Then, 1000 ml of a 30% aqueous solution of ethyl alcohol was added to dissolve the solid, followed by the addition of 500 ml of saturated NaCl aqueous solution. Then the pH was adjusted to 4 using a 0.2N aqueous solution of KH_2PO_4 and thereafter the solution was stirred and allowed to stand overnight. After removal of the formed precipitates by filtration, the filtrate was adjusted to pH 6.5 by the addition of saturated $NaHCO_3$ aqueous solution and concentrated under reduced pressure. The resulting solid was dissolved in 500 ml of ethyl alcohol, to which sodium sulfate was added, and the mixture was stirred. After filtration, the filtrate was concentrated and dried to give 9.2 g of a powder. 1 g of this ginkgo leaf extract was soluble into 100 ml of water. However, quantification revealed that the contents of flavone glycoside and terpene lactones were as low as 10.3% and 2.1%, respectively.

[0020]

[Effects of the Invention] As detailed supra, in accordance with the present invention, water-insoluble ginkgo leaf extract which was extracted with a water-containing organic solvent can be converted to a water-

soluble ginkgo leaf extract through an easy procedure, without losing two active ingredients, i.e., flavonoid glycoside and terpene lactones. Compared with the conventional processes, the process of the present invention, despite its simple procedure, achieves significantly high yield of conversion to a water-soluble ginkgo extract which contains sufficient amounts of both effective ingredients. The water-soluble ginkgo leaf extract obtained according to the present invention can be readily formulated into preparations for injections. Alternatively, various kinds of beverages and foods to which the ginkgo leaf extract is added can be produced easily. It is also possible to produce, with an ease, cosmetics and lotions containing the ginkgo leaf extract. Thus, the present invention can provide a wide range of products in which the ginkgo leaf extract is contained.